

L7 ANSWER 19 OF 27 CAPLUS COPYRIGHT 2003 ACS on STN  
AB The use of HPLC columns for the sepn. of proteins and nucleic acids is gradually increasing in biochem. labs. The efficiency of these columns for such sepns. has been much lower than that achievable for the sepn. of smaller mols. **Nonporous** microparticulate packings are the... logical answer one arrives at after consideration of the chromatog. behavior of proteins. **Nonporous** stationary phases are described for the sepn. of proteins, peptides, and nucleic acids. The stationary phases used are TSK-Gel NPR-C18. TSK-Gel NPR-DEAE, TSK-Gel NPR-SPs, and HYTACH MicroPell C18. A no. of fundamental properties of columns based on these sorbents were evaluated such as permeability, retention behavior towards small and large mols., load capacity, and stability. Instrumental requirements for these columns are discussed and some applications described.

AN 1989:570329 CAPLUS

DN 111:170329

TI Fast separation of biological macromolecules on **nonporous**, microparticulate columns

AU Rozing, Gerard P.; Goetz, Heinz

CS Waldbronn Anal. Div., Hewlett-Packard G.m.b.H., Waldbronn, D-7517/2, Fed. Rep. Ger.

SO Journal of Chromatography (1989), Volume Date 1988, 476, 3-19  
CODEN: JOCRAM; ISSN: 0021-9673

DT Journal

LA English

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L7 ANSWER 8 OF 27 CAPLUS COPYRIGHT 2003 ACS on STN  
AB For protein sepn. with high-performance ion-exchange chromatog., a monodisperse **non-porous** DEA (diethylamine)-silica gel was synthesized. Various factors influencing protein sepn., such as pH value of the mobile phase, salt concn., flow rate and NaCl gradient elution programming, were studied. The mass recoveries of proteins were measured. With a column of 50 mm long under gradient elution conditions, a mixt. of proteins consisting of albumin, bovine serum protein, insulin, .gamma.-globulins, RNase and lysozyme can be sepd. successfully in 15 min. Retention times of proteins are consistent with the general retention trend. This packing offers high column efficiency because of its smaller particle size. Thanks to its **non-porous** and hydrophilic nature, irreversible adsorption and denaturation would less probably happen. This packing is suitable for small-scale prepn. of high-purity proteins.

AN 1999:711092 CAPLUS  
DN 132:233803  
TI HPLC separation of proteins on a monodisperse **non-porous** silica gel anion-exchanger  
AU Lei, Xiaoling; Wang, Junde; Cong, Runzi  
CS Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116012, Peop. Rep. China  
SO Fenxi Ceshi Xuebao (1999), 18(4), 1-4  
CODEN: FCEXES; ISSN: 1004-4957  
PB Fenxi Ceshi Xuebao Bianjibu  
DT Journal  
LA Chinese

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E INSULIN/CN

L1 1 S E3

FILE 'CAPLUS' ENTERED AT 13:45:27 ON 19 AUG 2003

L2 276 S L1/BPN

L3 8157 S NONPOROUS OR NON(W) POROUS

L4 0 S L2 (P) L3

L5 2542699 S IMMOBIL? OR CARRIER? OR SUPPORT? OR POLYMER?

L6 46 S L2 AND L5

L7 27 S L1 AND L3

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U Graessel, Susanne; Roeling, Angelika; Hasilik, Andrej  
CS Inst. Physiol. Chem. Pathobiochem., Univ. Muenster, Muenster, D-4400, Fed.  
Rep. Ger.  
SO Analytical Biochemistry (1989), 180(1), 72-8

AU Schaumann, Christine; Oesch, Franz; Unger, Klaus K.; Wieser, Raimund J.  
CS Institut fuer Toxikologie, Johannes Gutenberg-Universitaet, Obere  
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SO Journal of Chromatography (1993), 646(1), 227-34

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# Analytical technique for studying the structure of glycoprotein N-glycans

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## ABSTRACT

The aim of this study was to develop an analytical strategy for the structural analysis of glycoprotein N-glycans by combining several sensitive methods without any elaborate equipment. The following consecutive steps were optimized and applied: (1) immobilization of glycopeptide N-glycosidase F (EC 3.2.2.18) on several polymeric and a silica support, the latter giving a maximum binding capacity of 11.3% of starting activity; (2) lectin affinity chromatography was miniaturized using Mobitec columns of volume 200  $\mu$ l; the binding capacity of glycoproteins on concanavalin A and wheat germ agglutinin columns was in the range 0.5–1  $\mu$ g; (3) N-linked oligosaccharides were isolated from commercial glycoproteins and from contactinhibin, a glycoprotein of  $M_r$  60 000–70 000. After derivatization with 8-aminonaphthalene-1,3,6-trisulphonic acid, they were separated by high-resolution electrophoresis.

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## INTRODUCTION

Glycans of cell membrane glycoproteins have gained increasing attention in recent years. Owing to their markedly different properties, they function, e.g., as adhesion mediators [1] or immunological markers [2] and are involved in complex processes such as development, differentiation and proliferation [3–5].

The N-linked carbohydrate moieties of con-

tactinhibin, a glycoprotein of  $M_r$  60 000–70 000, have been shown to be essential for its biological function, viz., contact-dependent inhibition of growth. It has been isolated from human embryonal lung fibroblasts [6], but has been identified in many different tissues of various species, especially in epithelia.

In order to obtain more relevant information about the structure–function relationship of the glycans, the analysis of their structure is essential. There are powerful methods available, but unfortunately they require sophisticated techniques and expensive instrumentation. High-pH anion-exchange chromatography with pulsed am-

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perometric detection [7] has been used for mapping of N-glycans [8], also in combination with fast atom bombardment mass spectrometry [9]. Another approach to characterizing the structures of glycans consists in the two-dimensional mapping of 2-pyridylaminated oligosaccharides using HPLC on an amide-modified and a reversed phase, respectively [10]. Combination with ionspray mass spectrometry further extends the potency of this method [11].

The aim of this work was to develop an analytical strategy which gives a maximum of information on the structural features of glycans without the use of elaborate equipment. In addition, the methods used should provide sufficient sensitivity to allow the analysis of plasma membrane glycans, which in most instances are available in only limited amounts. Further, the analysis should be rapid.

The procedure applied consisted in the following consecutive steps: the N-glycans were released enzymatically by glycopeptide N-glycosidase F, then they were separated according to discrete structural features by sequential lectin affinity chromatography (SLAC), followed by high-resolution electrophoresis of the glycans obtained.

This work focused on the three major aspects: (1) immobilization of the N-deglycosylating enzyme glycopeptide N-glycosidase F; the removal of the enzyme from the incubation mixture may be an essential requirement for the further analysis of both the protein and the glycan moieties; this can be achieved by the immobilization of the enzyme on a solid support; the immobilization of the enzyme is an effective means of reusing it, provided that the activity is preserved after incubation; (2) application of a miniaturized lectin affinity chromatographic system which is suitable for handling minute amounts of sample; (3) application of polyacrylamide gel electrophoresis of the fluorescence-labelled N-glycans isolated from standard glycoproteins and from concanavalin. This method combines the sensitivity of fluorescence detection with the power of electrophoretic separation according to the size and shape of the molecules [12,13].

## EXPERIMENTAL

### Chemicals

Pronase (Calbiochem, Bad Soden, Germany), Spectra/Por dialysis tubing (molecular mass cut-off 1000), ovalbumin, recrystallized five times (Serva, Heidelberg, Germany),  $\alpha$ -methyl-D-mannoside, N-acetyl-D-glucosamine,  $\alpha$ -methyl-D-glucoside, lactose, concanavalin A (Con A)-Sephacrose 4B, wheat germ agglutinin (WGA)-agarose, *Ricinus communis* agglutinin<sub>120</sub> (RCA<sub>120</sub>)-agarose (Sigma Chemie, Deisenhofen, Germany), immobilized papain (Pierce, Rockford, IL, USA), 8-aminonaphthalene-1,3,6-trisulphonic acid, disodium salt (ANTS) (Molecular Probes, Eugene, OR, USA), dextrin (Merck, Darmstadt, Germany) and Dowex 50W-X8, 200-400 mesh (Bio-Rad Labs., Munich, Germany) were used. Recombinant glycopeptide N-glycosidase F, EC 3.2.2.18, and resorufin-labelled glycopeptide were a generous gift from Dr. M. Watzele (Boehringer Mannheim, Tübingen, Germany). Recombinant glycopeptide N-glycosidase from *Flavobacterium meningosepticum* (25 000 U/mg protein; 1 U corresponds to the enzyme activity hydrolysing 1 nmol of dansylfetuin glycopeptide per minute at 37°C, pH 7.2) according to the manufacturer's information was without detectable activity of endoglycosidase F,  $\beta$ -galactosidase,  $\beta$ -glucosidase,  $\alpha$ - or  $\beta$ -mannosidase,  $\beta$ -N-acetylhexosaminidase,  $\alpha$ -L-fucosidase, sialidases or proteases.

Fetuin and asialofetuin (obtained by non-enzymatic desialylation according to ref. 14; information from manufacturer) were obtained from Boehringer (Mannheim, Germany). The other chemicals used were of the highest purity available.

### Equipment

A Model GE-2/4LS gel electrophoresis apparatus and an ECPS 2000/300 electrophoresis constant-power supply (Pharmacia-LKB, Uppsala, Sweden) and a UV light box (Bachofner, Reutlingen, Germany) were used. A Polaroid MP4 camera with a 667 film and a 58ES 090 5× red filter (Birmann und Weber, Bad Kreuznach,

Germany) was used with an exposure time of 10–40 s with an aperture  $f/4.5$ .

#### *Supports for immobilization*

LiChrospher Si 1000 was a gift from Merck. It was modified with isothiocyanatopropyltriethoxysilane (ITCPS) according to a procedure described elsewhere [15,16]. Eupergit C and Eupergit C1Z were obtained from Röhm (Darmstadt, Germany), Mobitec-Beads from Mobitec (Göttingen, Germany) and Biosynth from Riedel-de Haën (Seelze, Germany).

#### *Immobilization procedure*

Immobilization was performed according to the manufacturer's procedure. The concentration of the potassium phosphate buffer was varied between 0.1 and 1 M and the pH between 7.0 and 8.5. To obtain the acetylated glycopeptides ovalbumin (10 mg/ml in phosphate-buffered saline) was digested with pronase (1 mg/ml) for 18 h at 37°C. Pronase was inactivated at 100°C for 10 min. After cooling to room temperature, the solution was saturated with  $\text{NaHCO}_3$ . A 100- $\mu\text{l}$  volume of acetic anhydride per milligram of protein were added in 50- $\mu\text{l}$  portions at 10-min time intervals. After stirring for another 3 h, Dowex 50W-X8 was added until gas formation stopped. The solution was filtered over glass-wool, the pH was adjusted to 6–7 and the solution was dialysed (Spectra/Por) against distilled water, lyophilized and redissolved in the original volume of distilled water.

#### *Assay of immobilized glycopeptide N-glycosidase F*

A 2.5- $\mu\text{g}$  amount of resorufin-labelled  $\text{Man}_5$ -glycopeptide were added to the glycopeptide N-glycosidase sample to be tested in 300  $\mu\text{l}$  (250  $\mu\text{l}$  if solids were not present) of potassium phosphate buffer, (50 mM, pH 7.2)–20 mM EDTA and incubated for 20 min at room temperature. If immobilized enzyme was tested, the beads were removed by centrifugation and 250  $\mu\text{l}$  of the supernatant were transferred into a new vessel. Reaction was stopped by addition of 25 mg of Con A–Sephrose in 250  $\mu\text{l}$  binding buffer [50 mM potassium phosphate–25 mM NaCl (pH

7.8)] followed by incubation on a shaker for 15 min at 4°C. Glycopeptide bound to Con A beads were removed by centrifugation. The supernatant was mixed with an equivalent volume of 1 M  $\text{NaCO}_3$ . The fluorescence of the supernatant containing  $\text{Man}_5$ -free peptide and resorufin cleaved off by the activity of glycopeptide N-glycosidase was measured with excitation at 574 nm and emission at 584 nm emission against a blank obtained the same way without inclusion of enzyme. A sample containing neither enzyme nor Con A–Sephrose was used to measure maximum fluorescence.

#### *Sample preparation*

Contactinhibin was isolated from human embryonal lung fibroblasts FH109 as described previously [6]. Contactinhibin and laminin were digested with immobilized papain according to the protocol of the manufacturer. The N-glycans derived after digestion with glycopeptide N-glycosidase F of glycopeptides of contactinhibin and laminin and of the glycoproteins fetuin, asialofetuin and ovalbumin were derivatized with ANTS as described by Stack and Sullivan [13] without further purification.

#### *Serial lectin affinity chromatography*

For serial lectin affinity chromatography (SLAC) on lectin-agarose columns, 1-ml syringes were filled with 0.15 ml of lectin agarose. The columns could be run by hydrostatic pressure.

For the size-reduced system a special type of miniature columns. Compact Reaction columns (Mobitec), were used with a packing volume of 200  $\mu\text{l}$ . They had been modified with Con A and WGA by the manufacturer. Sample application and elution were done using pipettes and syringes, respectively. The binding buffer was 10 mM Tris-HCl–150 mM NaCl–1 mM  $\text{CaCl}_2$ –1 mM  $\text{MnCl}_2$ –0.02%  $\text{NaN}_3$ . The elution buffers were as follows: Con A, low affinity, 10 mM Tris-HCl–150 mM NaCl–10 mM  $\alpha$ -methyl-D-glucoside–0.02%  $\text{NaN}_3$ ; Con A, high affinity, 10 mM Tris-HCl–150 mM NaCl–0.5 M  $\alpha$ -methyl-D-mannoside–0.02%  $\text{NaN}_3$ ; WGA, 10 mM Tris-HCl–150 mM NaCl–0.5 M N-acetyl-D-glucosamine–0.02%  $\text{NaN}_3$ ; and RCAI, 10 mM

Tris-HCl-150 mM NaCl-0.5 M lactose-0.02% NaN<sub>3</sub>.

### Oligosaccharide polyacrylamide gel electrophoresis (O-PAGE)

Electrophoresis was performed according to Stack and Sullivan [13] with slight modifications. Briefly, the system consisted of a Laemmli Tris-glycine discontinuous buffer [17] without sodium dodecyl sulphate (SDS) and 2-mercaptoethanol, running gel (12 × 7.3 × 0.1 cm) with 25-30% total acrylamide (T) and 5% cross-linker (C) and a stacking gel with 5% T and 5% C. Gels were electrophoresed at a constant voltage of 200V for 15 min at 4°C and at 350V for 3-5 h until the front had reached the end of the gel or until it had migrated into the lower buffer reservoir, if a better resolution of high-molecular-mass glycans was to be obtained.

### O-PAGE standard

The maltooligosaccharide standard was obtained by acid hydrolysis of 0.25 g/ml dextrin in 0.3 M HCl at 95°C for 30 min followed by neutralization with NaHCO<sub>3</sub>.

## RESULTS AND DISCUSSION

### Immobilization of glycopeptide N-glycanase F

The major advantages of enzyme immobilization procedures are that (i) the solid-phase conjugates can be used several times and (ii) the enzyme can be removed from the reaction solution. Several different supports were applied for glycopeptide N-glycosidase F and were tested with respect to enzyme activity recovery and stability (Table I). The supports differ in the bulk composition, the reactive groups and spacers, the mean particle size and the porosity. The yield was calculated as a percentage of the activity of the immobilized enzyme in relation to the activity of the amount of soluble enzyme employed.

The best results were achieved with ITCPS-activated LiChrospher Si 1000 beads (Fig. 2). The recovery of 11.3 ± 4.8% of the initial activity employed was obtained by adding acetylated glycopeptides, which were prepared by a pronase digest of ovalbumin, to the incubation solution. Without adding these peptides the recovery dropped to 6.3 ± 3.0%. This may be explained by a protecting effect of the glycopeptides.

TABLE I  
ACTIVATED SUPPORTS USED FOR IMMOBILIZATION OF GLYCOPEPTIDE N-GLYCOSIDASE F AND ACTIVITY YIELDS

Trade name*	Bulk composition	Mean particle diameter, $d_p$ (μm)	Activation	Enzyme activity ± standard deviation (%)
LiChrospher Si 1000	Silica	ca. 10	Isocyanate	11.3 ± 4.8
Mobitec-Beads	Cross-linked organic polymer	ca. 20	Isocyanate	3.05 ± 2.1
Eupergit C1Z	Methacrylamide + bismethacrylamide copolymer	0.6-1.4	Epoxy	1.1 ± 2.4
Eupergit C	As Eupergit C1Z	ca. 150	Epoxy	0
Biosynth 1	Vinyl acetate + divinylethylene-urea copolymer	20-40	Epoxy	0.74 ± 1.9
Biosynth 2	As Biosynth 1	100-200	Epoxy	0.16 ± 0.21

\* For suppliers, see Experimental.

Acetylation prevents them from being coupled to the support so that they can bind to the active site of the enzyme. Hence the active site is sterically protected, leading to a lower statistical probability of this region of the enzyme being inactivated by interacting with the reactive groups.

A similar protecting mechanism is observed by immobilization of lectins in the presence of their hapten-carbohydrates, which leads to increased binding capacities of the lectins [18,19].

All other materials except LiChrospher Si 1000 show low recoveries of enzyme activity. LiChrospher Si 1000 mainly differs from the other materials in bulk composition and reactive groups. Experimental data are not yet available, but its better suitability for the immobilization of glycopeptide N-glycanase F [20] may be due to interactions between protein and silica that do not occur with organic cross-linked polymers, or to the reactive isothiocyanate group.

#### Compact reaction columns for affinity chromatography

Lectin affinity chromatography (LAC) is a widely used method for the separation and characterization of glycoproteins, both with lectins bound to agarose and on microparticulate rigid supports [21,22]. When only microgram amounts of glycoproteins are available, the relatively large volumes required for washing and elution steps lead to significant losses of both sensitivity and sample, so that size reduction of the LAC system is essential. The Compact Reaction columns (CR columns), which fit into 1.5-ml reaction caps, have been found to be best suited for miniaturization. On these columns 10–13  $\mu\text{g}$  each of the lectins Con A and WGA were immobilized. The binding capacity was evaluated with tritium-labelled fetuin (cf., Fig. 1) and horseradish peroxidase, which specifically bind to WGA and Con A, respectively.

The binding capacity ranged between 0.5 and 1  $\mu\text{g}$  of glycoprotein. The bound protein was eluted with 0.2–0.3 ml of elution buffer. The main peak was contained in 0.1 ml. Enzyme assays and radiometric labelling were successfully applied as detection methods.

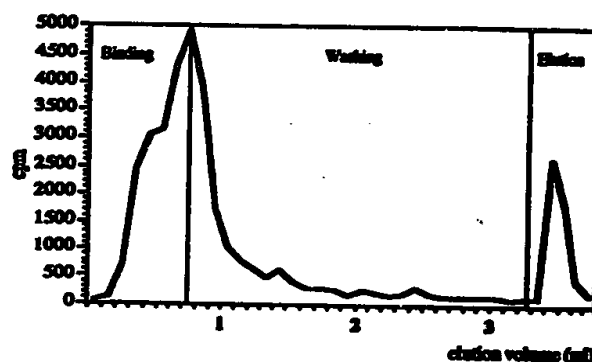


Fig. 1. Typical chromatogram of the binding, washing and elution of tritium-labelled fetuin ( $2 \mu\text{g/ml}$ ,  $32 \cdot 10^3 \text{ cpm}/\mu\text{g}$ ) on a WGA CR column. Binding and washing buffer, 10 mM Tris-HCl-150 mM NaCl-1 mM  $\text{CaCl}_2$ -0.02%  $\text{NaN}_3$ -6 mM 3-[3-cholamidopropyl]dimethylammonio-1-propanesulphonate. Elution buffer, 10 mM Tris-HCl-150 mM NaCl-0.5 M N-acetyl-D-glucosamine-0.02%  $\text{NaN}_3$ .

#### Oligosaccharide PAGE

Derivatization with a charged component is an essential step for the electrophoresis of carbohydrates. Reductive amination with ANTS is selective for reducing carbohydrates, introduces negative charges and, as a fluorophore, permits sensitive detection. Similarly to the SDS-PAGE of proteins, the oligosaccharides were separated in an electric field according to their molecular mass, structure and shape. Owing to the three negative charges of ANTS separation occurs faster than with mono- or disulphonic acids as coupling reagents. The detection limit was determined to be 10 pmol of ANTS and, with ovalbumin as a standard, 25  $\mu\text{g}$  or 0.6 nmol of glycoprotein, using the described equipment. The dextrin hydrolysate was used as a marker to calculate the relative migration of unknown oligosaccharides, expressed in glucose units as degree of polymerization (d.p.). The calibration graph is shown in Fig. 2 (25% T, 5% C).

The N-glycans released from fetuin gave five or six major and some minor bands, depending on the amount loaded on the gel and on the exposure time (Fig. 3). Among the N-glycans of asialofetuin a very intense band appeared, which migrated more slowly than the major fetuin bands. Most likely this behaviour is due to the missing charge of the removed sialic acid. These

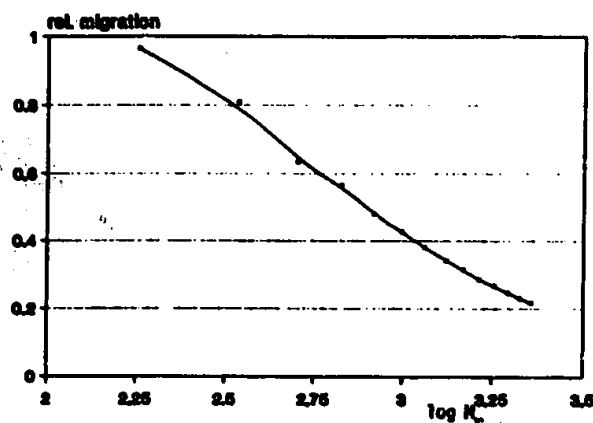


Fig. 2. Calibration graph for ANTS oligosaccharides on a 25%T-5%C polyacrylamide gel. Relative migration is calculated in relation to the fluorescent front of excess ANTS.

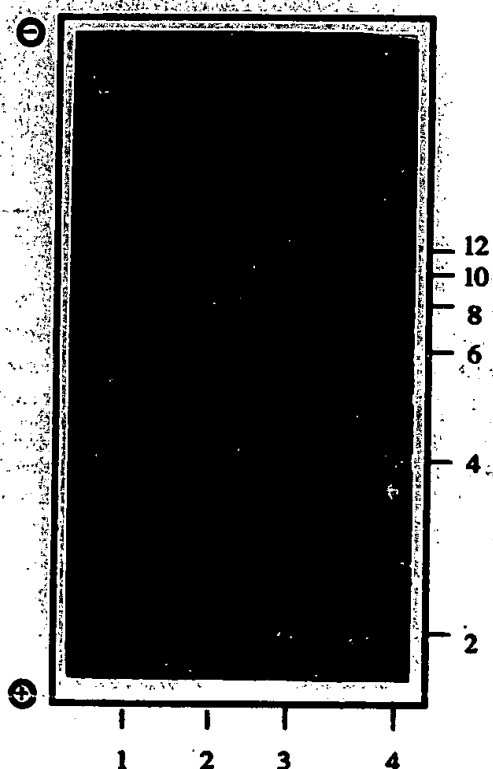


Fig. 3. Oligosaccharide-PAGE of N-linked ANTS oligosaccharides derived from fetuin, asialofetuin and ovalbumin on a 30%T-5%C polyacrylamide gel. The ANTS oligosaccharides prepared from the dextrin hydrolysate served as markers. The lanes contain ANTS oligosaccharides derived from 200  $\mu$ g of fetuin and ovalbumin, 300  $\mu$ g of asialofetuin and 0.2-1 mg of dextrin hydrolysate.

results are in agreement with the data described by Stack and Sullivan [13].

For ovalbumin nine major oligosaccharide structures of the high-mannose and the hybrid types have been reported [23,24]. The ANTS oligosaccharides from ovalbumin were separated into eight bands, three of them having higher intensity than the others (Fig. 3). A number of additional minor bands with lower d.p. were detected at higher concentrations.

Contactinhibin showed five major bands of ANTS oligosaccharides (Fig. 4), which were calculated to have d.p. 8, 7.5, 6.6, 6.1 and 4.7. Two of these values correspond to the  $R_f$ -values of the standard N-linked oligosaccharides given by Stack and Sullivan [13]. One is a fucosylated biantennary structure with terminal GlcNAc (d.p. 6.6, 27% T) and the other corresponds to a biantennary structure with two terminal galactoses (d.p. 7.5, 27% T). These standard N-linked oligosaccharides have been prepared from glycoproteins. Further studies on the structure and function of the carbohydrate moiety of contactinhibin are in progress.

Finally, SLAC and O-PAGE were combined.

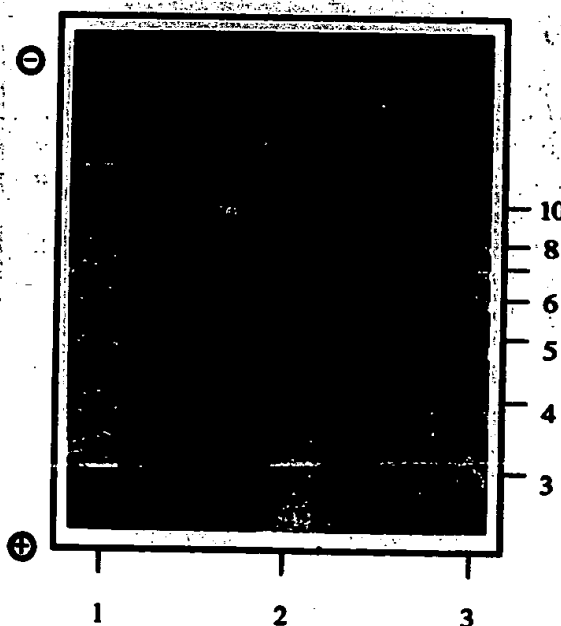


Fig. 4. Oligosaccharide-PAGE of ANTS-labelled N-linked glycans of contactinhibin on a 27%T-5%C polyacrylamide gel prepared from 150-200  $\mu$ g of protein. ANTS-dextrin hydrolysate as in Fig. 3.

Laminin, a glycoprotein involved in cell adhesion [25], was used as a model glycoprotein in this study. It is a high-molecular-mass glycoprotein with a large number of differing N-glycans, which can be fractionated, e.g., by lectin affinity chromatography on a Con A column [26-28]. The first step involved SLAC of the N-glycans on the Con A column. The unretarded fraction was applied directly to the WGA column and the unbound fraction from this column was subsequently added to the RCA<sub>120</sub> column. After elution of the unbound fraction from the RCA<sub>120</sub> column, the retained glycans were eluted from each lectin column with a specific buffer.

Fig. 5 shows the results of the combination of SLAC and O-PAGE. The complete pattern of laminin N-glycans is shown in lane 2. The separation by SLAC resulted in five fractions. The main portions consisted in the unretarded fraction (lane 3), which contains, e.g., complex types of tri- or higher antennary type glycans, and the fraction with low affinity to Con A (lane 4),

containing biantennary complex-type N-glycans [29,30]. This is in accord with results described by other groups [26-28]. High-mannose or hybrid-type N-glycans have a high affinity to Con A (lane 5) [29,30]. The WGA fraction (lane 6) contains hybrid-type glycans or complex-type glycans with bisecting N-acetylglucosamine [31,32] and the RCA<sub>120</sub> fraction (lane 7) contains N-glycans with terminal galactose [33,34].

## CONCLUSIONS

An analytical strategy applicable in any laboratory with facilities for protein analysis was established and was successfully applied to the analysis of the N-glycans of contactinhibin, a plasma membrane glycoprotein available in only minute amounts. Five major N-glycans with d.p. 8, 7.5, 6.6, 6.1 and 4.7 glucose units were found, and in addition oligosaccharides with higher (d.p. between 12 and 15) and lower molecular mass (d.p. 5 and lower) were also observed.

O-PAGE gives evidence about the heterogeneity, the relative amounts and the size of the N-glycans present in the glycoprotein. In order to elucidate further the glycan structures, additional methods may be used, e.g., lectins with other specificities such as *Phaseolus vulgaris* agglutinin, *Maackia amurensis* agglutinin or sequential exoglycosidase digestion followed by the techniques described in this paper.

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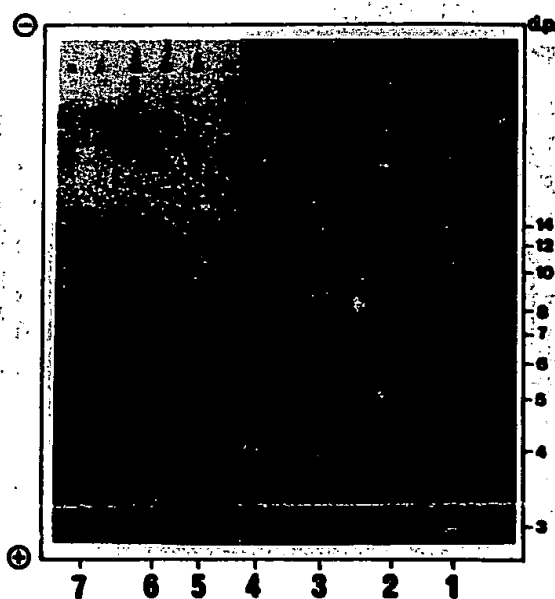


Fig. 5. Electrophoretic resolution of fractions of laminin derived ANTS oligosaccharides after SLAC. A 200  $\mu$ g amount of laminin was used as starting material. Polyacrylamide gel: 25%T-5%C. Lanes: 1 = ANTS oligosaccharide standards; 2 = unfractionated laminin N-glycan pattern; 3 = unretarded fraction; 4-7 = eluates of the lectin affinity columns (4 = Con A, low affinity; 5 = Con A, high affinity; 6 = WGA; 7 = RCA<sub>120</sub>).

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